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6008890001

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United Kingdom

4. Title of the invention

CULTURE OF MICROORGANISMS FOR THE SYNTHESIS OF A POLYUNSATURATED FATTY ACID

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CULTURE OF MICROORGANISMS FOR THE SYNTHESIS OF A POLYUNSATURATED FATTY ACID

The invention relates to a method of culturing a microorganism for the synthesis of a polyunsaturated fatty acid, particularly docosahexaenoic acid, by the microorganism. The invention also relates to oils and preparations of fatty acids, particularly docosahexaenoic acid, prepared from microorganisms cultured in accordance with the method.

Such a method is known from EP 0515460 which describes the culture of the dinoflagellate *Crypthecodinium cohnii* (*C. cohnii*) with glucose. The *C. cohnii* uses the glucose as the carbon source and synthesises docosahexaenoic acid.

According to a first aspect of the invention there is provided a method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionised form of an acidic group, the microorganism using the species as a carbon source and synthesising docosahexaenoic acid.

The use of such a species as a carbon source may allow the production of comparable or greater amounts of docosahexaenoic acid, for given culture conditions, compared to culture methods that do not use such a species. Additionally, by choosing suitable,

cheap sources of such species, it may be possible to produce docosahexaenoic acid more cheaply. Further, in the culture method described in EP 0515460, it is necessary to impose a stationary phase in order to achieve satisfactory production of docosahexaenoic acid. Such a phase may not be necessary when such a species is provided as a carbon source.

According to a second aspect of the invention there is provided a method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionised form of an acidic group, the microorganism synthesising docosahexaenoic acid containing carbon from the species.

According to the third aspect of the invention there is provided a method of culturing a microorganism for the synthesis of a polyunsaturated fatty acid by the microorganism, comprising culturing *C. cohnii* with an organic species comprising an acidic group or an ionised form of an acidic group, the *C. cohnii* using the species as a carbon source and synthesising a polyunsaturated fatty acid.

The following is a more detailed description of embodiments of the invention, by way of example, reference being made to the appended drawings in which:

Figure 1 is a schematic representation of part of a fermentation apparatus;

Figure 2 is a graph showing certain parameters over time during culture of a first microorganism;

Figure 3 is a similar graph to Figure 2 showing the parameters during culture of a second microorganism;

Figure 4 is a similar graph to Figure 2 showing the parameters during culture of a third microorganism;

Figure 5 is a graph showing dry cell weight over time during culture of the first microorganism after different pretreatments;

Figure 6 is a graph showing lipid produced over time during culture of the first microorganism after the pretreatments of Figure 5;

Figure 7 is a graph showing dry cell weight over time during culture of the first microorganism under different conditions;

Figure 8 is a graph showing lipid production over time during culture of the first

microorganism under the different conditions of Figure 7; and

Figure 9 is a similar graph to Figure 8 in which lipid production is expressed in a different manner.

Example 1

In Example 1, six microorganisms are cultured, separately, in a growth medium to which an organic acid is added. The microorganisms synthesise docosahexaenoic acid containing carbon from the organic acid.

The culture method will be described in detail for one of the six microorganisms - the strain of *C. cohnii* available from the American Type Culture Collection and identified by the number 30772 (*C. cohnii* ATCC 30772). The remaining five microorganisms are also strains of *C. cohnii* available from the American Type Culture Collection. They are identified, respectively, by the numbers: 30541; 50298; 40750; 30555; and 30557. These five strains of *C. cohnii* are cultured in an identical manner to *C. cohnii* 30772 and their culture will not be described in detail. Other strains which are also available from the ATCC or from other sources would be expected to behave similarly to those described here.

The culture of *C. cohnii* 30772 is performed in a 5 L fermenter 10 of known type (see

Figure 1). The fermenter 10 is provided with a pH electrode 11 that is positioned in the fermenter 10 for monitoring the pH of the growth medium in the fermenter 10. The pH electrode 11 is connected via a control device 12 to a pump 13. The control device 12 is programmed to cause the pump 13 to pump a liquid from a reservoir 14 into the fermenter 10 when the pH electrode 11 detects a pH greater than a predetermined value. This process is described in greater detail below.

The fermenter 10 is also provided with a thermometer (not shown) that is positioned for monitoring the temperature of the growth medium (and also the *C. cohnii* 30772) in the fermenter 10. The thermometer is connected via a control device (not shown) to a heater (not shown). The control device is programmed to control the heater on the basis of the temperature determined by the thermometer so as to maintain the growth medium (and the *C. cohnii* 30772) at a desired temperature within the fermenter 10.

The fermenter 10 is also provided with an aerator (not shown) that is connected to a source of air (not shown) for aerating the growth medium within the fermenter 10. An oxygen sensor (not shown) is positioned for measuring dissolved oxygen concentration in the growth medium within the fermenter 10. A stirrer (not shown) is provided for stirring the growth medium (and the *C. cohnii* 30772). The oxygen sensor is connected to the stirrer by a control device (not shown) for varying the

speed of the stirrer in response to the oxygen concentration detected by the oxygen sensor so as to maintain the oxygen concentration at a desired level.

Before initiating the culture, the *C. cohnii* 30772 used to inoculate the fermenter 10 (the "inoculum") is prepared from a standing culture of this organism using a pre-inoculation medium. The pre-inoculation medium contains (initially) glucose (27 g/L), yeast extract (3.8 g/L) and sea salts (25 g/L - commercial preparation purchased from SIGMA, Dorset, UK). Before use, the pH of the pre-inoculation medium is corrected to 6.5 by the addition of 1M KOH and the medium is autoclaved at 121°C for 30 minutes (the glucose is sterilised separately to avoid caramelisation).

The inoculum is prepared by adding 10 ml of the standing culture (4 days old) to a 250 ml flask containing 100 ml of the pre-inoculation medium. The flask is incubated at 27°C while shaking at 200 rpm for 3 days. 70 ml of the incubated mixture is then added to a 2 L flask containing 700 ml of pre-inoculation medium. The 2L flask is then incubated at 27°C while shaking at 200 rpm for 3 days after which the full content of the 2 L flask forms the inoculum.

The growth medium used for culture in the fermenter 10 contains (initially) sodium acetate (1 g/L), yeast extract (7.5 g/L) and sea salts (25 g/L). Before use, the pH of the growth medium is corrected to 6.5 by the addition of 1M KOH and the medium

is autoclaved at 121°C for 1 hour.

Glacial acetic acid mixed with water to give a 50% (v/v) solution is added to the reservoir 14.

The culture is initiated by adding the inoculum into the fermenter 10. The total volume in the fermenter 10 is made up to 3.5 L with growth medium. The fermenter 10 is then closed.

The control device connected to the thermometer and the heater is set so that the heater maintains the growth medium and the *C. cohnii* 30772 at 27°C.

The aerator is set to aerate the growth medium at a rate of 1 volume of air per volume of medium per minute. The control device connected to the oxygen sensor and the stirrer is set so that the dissolved oxygen concentration is maintained above 20% of air saturation. In order to achieve this, the speed of the stirrer varies between 300 to 1000 rpm.

Under these conditions, the *C. cohnii* 30772 grows readily. As the *C. cohnii* 30772 grows it uses the acetate from the growth medium as a carbon source. In fact, it is believed that the *C. cohnii* 30772 takes up acetic acid formed from the acetate - acetic

acid being formed in accordance with the equilibrium reaction: acetate + $H^+ \rightleftharpoons$ acetic acid. Acetate is, of course, a carboxylate ion - it comprises the ionised form of the carboxylic acid group. As the cells use the acetate/acetic acid, the pH of the growth medium increases. As indicated above, the pH of the growth medium is monitored by the pH electrode 11 and when the pH exceeds a pre-set value (slightly greater than 6.5), the control device 12 causes the pump 13 to pump acetic acid solution from the reservoir 14 to the fermenter 10 where it mixes with (and is incorporated into) the growth medium. The addition of the acetic acid solution reduces the pH of the growth medium. The arrangement is such that the pH of the growth medium remains at about 6.5 during the culture.

Acetic acid added in this way dissociates to form, in equilibrium with the acid as indicated above, acetate and protons. The acetic acid derived from the added acetic acid solution, and the acetate formed from this acetic acid, are used as a carbon source by the *C. cohnii* 30772. As indicated above, it is believed that it is acetic acid that is actually take up by the *C. cohnii* 30772. However acetate may also be taken up (if it is not, it is used as a carbon source by first being converted to acetic acid). The use of the acetic acid/acetate (derived from the added acetic acid) by the *C. cohnii* similarly leads to an increase in the pH of the growth medium and this is counteracted by the addition of more acetic acid - so as to maintain the pH at about 6.5 - as described above.

By adding acetic acid over a period of time, a considerable amount of acetic acid/ acetate can be provided as a carbon source for the *C. cohnii* 30772 while, at any particular moment in time, the concentrations of the acetate and the acetic acid are sufficiently low so as not to be detrimental to growth of the *C. cohnii* 30772. The system of adding acetic acid in response to an increase in pH caused by use of acetic acid/acetate is a convenient way of providing acetic acid/acetate as it is needed to replenish acetic acid/acetate used by the microorganisms. The addition of acetic acid in this way allows optimum growth of the *C. cohnii* 30772, a high final biomass (consisting of cells of *C. cohnii* 30772), a high content of oil in the cells and a high proportion of docosahexaenoic acid within the oil.

The acetic acid/acetate is the sole or main carbon source for the *C. cohnii* 30772 - small amounts of other carbon sources may be provided in the yeast extract.

The yeast extract present in the growth medium is provided, primarily, as a source of nitrogen, essential vitamins, amino acids and growth factors. The sea salts are needed for osmo-protection of the *C. cohnii* 30772, which are marine cells in origin.

If, during the culture, the pH falls below 6.5, the pH can be brought back to 6.5 by adding NaOH (2M). This can be done automatically.

During the culture method, samples of the *C. cohnii* 30772 together with growth medium are taken at time intervals. Each sample is analysed to determine dry cell weight, lipid content, docosahexaenoic acid content, and polysaccharide content. The results of these analyses are shown in Figure 2 which shows that dry cell weight, lipid content, docosahexaenoic acid content and polysaccharide content increase with time over the course of the culture method. Significantly, Figure 2 shows that docosahexaenoic acid forms a substantial part of the lipid synthesised by the *C. cohnii* 30772.

As the acetic acid/acetate is a carbon source, the docosahexaenoic acid will contain carbon derived from the acetic acid/acetate.

Growth of *C. cohnii* 30772 proceeds until the cells reach their maximum biomass yield (after about 140 hours of culture). This is marked by the fact that the pump 13 ceases to operate, indicating that the *C. cohnii* 30772 is no longer consuming acetic acid/acetate. At this stage, no further production of lipid (including docosahexaenoic acid) will occur.

At this stage, the *C. cohnii* 30772 cells and the growth medium are removed from the fermenter 10 and the biomass (cells of *C. cohnii* 30772) is separated. The oil content of the biomass is extracted using known methods. This oil contains

docosahexaenoic acid and is useful as a food supplement. The docosahexaenoic acid may be partially or totally purified from the oil.

Table 1 shows dry cell content, lipid content and the content of docosahexaenoic acid expressed as a percentage of the total fatty acid content of the lipid, as measured after *C. cohnii* 30772 has ceased to grow. Dry cell content and lipid content are expressed as g per L of growth medium and biomass. Table 1 also shows the maximum lipid content in the cells. This is the highest value of lipid content per dry cell weight over the course of the culture. Additionally, Table 1 shows the same parameters measured after culturing the other five strains of *C. cohnii* (strain Nos. 30541, 50298, 40750, 30555, and 30557) under identical conditions.

Table 1: Growth of six different strains of *Crypthecodinium cohnii* obtained from the American Type Culture Collection (ATCC) on acetate/acetic acid as described in Example 1.

	Values after 140 h of growth			
Strain (ATCC no)	Dry cells (g/L)	Lipid (g/L)	DHA in total fatty acid from lipid (%)	Maximum lipid in cell (g lipid/ 100g cells)
30772	17	7.48	59	44
30541	45.5	8.32	33	24
50298	45.5	12.3	31	30
40750	17	3.31	46	22
30555	20.7	2.8	30	15
30557	25.6	4.7	40	18

DHA - Docosahexaenoic acid

Table 2 shows further measured parameters and derived values relating to the end points (140 hours of growth) of cultures of the six strains of *C. cohnii*. Specifically, Table 2 shows the amounts of acetic acid utilised and the final volumes of the cultures. Table 2 also shows the yields of biomass, docosahexaenoic acid and lipid.

Table 2: Yields of biomass, lipid and DHA of six different strains of *C. cohnii* obtained from the American Type Culture Collection (ATCC) on acetate/acetic acid as described in Example 1.

Strain ATCC no	Acetic acid utilised/ Final volume	Yields of Biomass (g/g acetic acid utilised)	Yields of DHA (g/g acetic acid utilised)	Yields of Lipid (g/g acetic acid utilised)
30772	607 g/ 4.2 L	0.118	0.03	0.05
30541	1039 g/ 4.6 L	0.2	0.012	0.037
50298	1107 g/ 5.2 L	0.21	0.018	0.06
40750	340 g/ 4.5 L	0.22	0.026	0.044
30555	498 g/ 4.2 L	0.18	0.007	0.024
30557	754 g/ 4.7 L	0.16	0.01	0.029

As will be seen from Tables 1 and 2, there is a considerable variation in the production and yields of dry cells/biomass, lipid and docosahexaenoic acid between the different strains of *C. cohnii*. Each of these strains, however, produces potentially useful amounts of docosahexaenoic acid.

Figures 3 and 4 show the same parameters as those shown in Figure 2 - during the

culture of *C. cohnii* 30541 and 50298, respectively. The results are similar to those with *C. cohnii* 30772.

It will be appreciated that the culture method of Example 1 constitutes a convenient method for the production of docosahexaenoic acid containing biomass. Moreover, this method may produce comparable or greater amounts of docosahexaenoic acid compared to methods in which glucose is used as the sole carbon source.

It will be appreciated that the method of Example 1 may be varied.

For example, additional nutrients could be added to the acetic acid solution in reservoir 14 so that the nutrients are added to the fermenter 10 together with the acetic acid. Suitable additional nutrients comprise, for example, sources of nitrogen, sources of phosphorus, vitamins and additional salts. Alternatively, instead of adding the nutrients directly to the acetic acid solution in the reservoir 14, the nutrients could be provided in the form of a separate solution in a separate reservoir. Nutrients from the separate reservoir would be pumped into the fermenter 10 by a separate pump. The separate pump would be controlled by the control device 12 and may, for example, operate at the same time as the pump 13 operates. If additional nutrients are added using a separate pump, the separate pump may be operated to add the nutrients during part, but not all, of the culture period.

Additional carbon source compounds could be added together with the acetic acid. Suitable compounds comprise, for example, oils and lipids containing various precursors of docosahexaenoic acid.

The acetic acid used could be industrial grade or may be produced by other chemical processes either as a primary or a secondary product. The presence of other aliphatic acids within the acetic acid solution would not be deleterious and may provide additional carbon sources. Waste solutions from industrial processes comprising acetic acid together with other compounds may be suitable for use in place of the acetic acid solution in reservoir 14.

Instead of acetic acid/acetate, other organic species comprising an acidic group or an ionised form of an acidic group, or mixtures of such species, can be used as a carbon source. Preferably, the carbon source is a carboxylic acid or a carboxylate ion, or mixtures of these species. If the carbon source is provided by adding an organic acid to the growth medium, then, preferably the utilisation of the organic acid (and/or its ionised form) by the microorganism results in an increase in the pH of the growth medium.

The volume of the inoculum may vary. Preferably, this volume will be between 1% and 20% of the initial volume of the culture mixture. More preferably, the volume

will be between 5 and 10% of the initial volume of the culture mixture.

Whereas the method of Example 1 is a batch process, it will be appreciated that the culture method may be adapted to be performed as a continuous or semi-continuous process. In this case, the acetic acid and other compounds needed for cell growth, cell maintenance and for oil production would be added into the fermenter throughout the course of the culture. Cells and spent medium would be removed from the fermenter either continuously during the course of the culture or from time to time depending on the volume of the culture or density of the cells. Unlike the culture method described in EP 0515460, which uses glucose as an apparently sole carbon source, the use of acetic acid/acetate as a carbon source does not require the imposition of a stationary phase in order to cause satisfactory production of docosahexaenoic acid. This is particularly advantageous as it will facilitate adaptation of the method to be a continuous or semi-continuous process.

Although the method of Example 1 is performed in a 5 L fermenter 10, it will be appreciated that the method can be performed in larger, industrial scale fermenters. When culture is performed in larger fermenters, it may be advantageous to add the acetic acid at a plurality of different points into the fermenter. This would aid dispersion of the acid and avoid build-up of any local high concentration of acid.

Whereas, in the method of Example 1, the oxygen concentration is controlled by controlling the stirring speed, it will be appreciated that oxygen concentration may be controlled by controlling the aeration rate or by controlling both aeration rate and stirring speed.

The configuration of the fermenter 10 may be changed and an air-lift fermenter, or a fermenter having appropriate aeration and mixing without mechanical stirring, may be used.

Microorganisms other than *C. cohnii* may be used. When *C. cohnii* is used, it may be used to synthesise other polyunsaturated fatty acids instead of, or in addition to docosahexaenoic acid.

The initial concentration of sodium acetate in the growth medium may be varied. It may be between 0.5 g/L and 10 g/L. Preferably, it will be between 1 g/L and 5 g/L.

The pH of the growth medium may be maintained at values other than 6.5 during the culture. The pH may be maintained at values between 5 and 8. Preferably, the pH is maintained between 6 and 7. The acceptable and preferred pH values will depend on the microorganism used.

Example 2

In the method of Example 2, *C. cohnii* 30772 is grown, prior to culture in the fermenter 10 as described above in Example 1, with acetic acid/acetate. It is hypothesised that this will allow the cells to adapt to acetic acid/acetate so that they will grow more quickly during culture in the fermenter 10.

The method of Example 2 is similar to the method of Example 1 with the exception of the preparation of the inoculum. The inoculum is prepared by adding 10 ml of the standing culture of *C. cohnii* 30772 to a 250 ml flask containing 100 ml of the pre-inoculation medium described above in Example 1. The flask is incubated at 27°C while shaking at 200 rpm for 3 days. After 3 days, 70 ml of the incubated mixture is added to a 1 L fermenter and the volume in the 1 L fermenter is made up to 700 ml with the growth medium described above in Example 1. The 1 L fermenter is then closed and the fermenter is heated and aerated in the same manner as the 5 L fermenter 10 of Example 1. Additionally, acetic acid is added in response to increases in pH in the same manner as described in Example 1. The *C. cohnii* 30772 is allowed to grow in the 1 L fermenter for 72 hours after which the content of the 1 L fermenter forms the inoculum.

After this period, 700 ml of inoculum is taken from the 1 L fermenter and cultured with the growth medium of Example 1 in the 5 L fermenter 10, exactly as described

above in Example 1. During the culture in the 5 L fermenter, samples are taken at time intervals for analysis of dry cell weight and lipid content. The results of these analyses are shown in Figures 5 and 6, respectively. For comparison, Figures 5 and 6 also show comparable analyses on samples taken during the culture of *C. cohnii* 30772 during Example 1. As will be seen from Figures 5 and 6, both dry cell weight and lipid content increase more rapidly during culture in the 5 L fermenter 10 when the *C. cohnii* 30772 has been pre-grown with acetic acid/acetate (in Example 2), as opposed to being pre-grown with glucose as a carbon source (in Example 1).

Example 3

In Example 3, *C. cohnii* 30772 is grown with acetic acid/acetate in the same manner as in Example 1, and with different concentrations of sea salts.

A first inoculum of *C. cohnii* 30772 is prepared as described above in Example 2. The first inoculum is then cultured in the 5 L fermenter 10, exactly as described in Example 1.

A second inoculum is prepared in the same way as the first inoculum. The second inoculum is then cultured in the 5 L fermenter 10, as described in Example 1, but with an initial concentration of sea salts in the growth medium of 16 g/L (rather than 25 g/L in Example 1).

During the culture of the first and second inocula, samples are taken at time intervals for the analysis of dry cell weight and lipid content. The results of these analyses are shown in Figures 7, 8 and 9. As will be seen from these Figures, culture of the *C. cohnii* 30772 with 16 g/L sea salts results in poorer lipid production compared to culture with 25 g/L sea salts.

CLAIMS

1. A method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionised form of an acidic group, the microorganism using the species as a carbon source and synthesising docosahexaenoic acid.

2. A method according to claim 1, wherein the microorganism is a dinoflagellate or a genetically modified variant thereof.

3. A method according to claim 2, wherein the microorganism is *Crypthecodinium cohnii* or a genetically modified variant thereof.

4. A method according to any one of claims 1 to 3, wherein the species is a carboxylic acid or a carboxylate ion.

5. A method according to claim 4, wherein the species is acetic acid or acetate.

6. A method according to any one of claims 1 to 5, wherein the species is the main carbon source for the microorganism during the culture of the microorganism.

7. A method according to any one of claims 1 to 6, wherein the microorganism is cultured in a medium, an amount of the species being provided in the medium over a period of time during the culture of the microorganism.

8. A method according to claim 7, wherein the use of the species as a carbon source by the microorganism causes an increase in pH of the medium, said provision of the amount of the species comprising addition of an organic acid to the medium in response to the increase in pH so as to decrease the pH of the medium.

9. A method according to claim 8, wherein the organic acid is the species.

10. A method according to claim 8, wherein the organic acid ionises to form the species.

11. A method according to any one of claims 8 to 10, wherein the organic acid is added so as to maintain the pH substantially at a desired value.

12. A method according to claim 11, wherein the desired value is pH 6.5.

13. A method according to any one of claims 8 to 12, wherein the pH of the medium is monitored by means that produces a signal that is used to control the

addition of the organic acid to the medium.

14. A method according to claim 13, wherein the signal is used to control addition of one or more of a nitrogen source, a phosphorus source, an amino acid, a vitamin, a salt or another growth factor during the culture of the microorganism.

15. A method according to any one of claims 8 to 13, wherein the organic acid is added to the medium in a mixture comprising a further compound.

16. A method according to claim 15, wherein the further compound is a further organic acid.

17. A method according to claim 15, wherein the further compound is a lipid.

18. A method according to any one of claims 15 to 17, wherein the mixture is a waste product from an industrial process.

19. A method according to claim 15, wherein the further compound is a nitrogen source, a phosphorus source, an amino acid, a vitamin, a growth factor, a salt or a lipid.

20. A method according to any one of claims 1 to 19, wherein prior to said culture with said species, the microorganism is grown with said species.

21. A method according to any one of claims 1 to 20, wherein the microorganism is cultured with an organic nitrogen source, preferably with yeast extract.

22. A method according to any one of claims 1 to 21, wherein the microorganism is cultured with salts or osmoticants, preferably with sea salts.

23. A method according to any one of claims 1 to 22, wherein said culture is performed as a batch process or a fed-batch process.

24. A method according to any one of claims 1 to 22, wherein said culture is performed as a continuous process or semi-continuous process.

25. A method according to any one of claims 1 to 24, wherein the method further comprises extracting lipids including docosahexaenoic acid from the microorganism.

26. A method according to any one of claims 1 to 25, wherein the method further comprises the purification or partial purification of docosahexaenoic acid from the microorganism.

27. A method according to any one of claims 1 to 26, wherein the culture does not include a stationary phase.

28. An oil comprising docosahexaenoic acid prepared from a microorganism cultured in accordance with any one of claims 1 to 27.

29. An at least partially purified preparation of docosahexaenoic acid prepared from a microorganism cultured in accordance with any one of claims 1 to 27.

30. A method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionised form of an acidic group, the microorganism synthesising docosahexaenoic acid containing carbon from the species.

31. A method of culturing a microorganism for the synthesis of a polyunsaturated fatty acid by the microorganism, comprising culturing *C. cohnii* with an organic species comprising an acidic group or an ionised form of an acidic group, the *C. cohnii* using the species as a carbon source and synthesising a polyunsaturated fatty acid.

32. An oil comprising said polyunsaturated fatty acid of claim 31, prepared from

a microorganism cultured in accordance with claim 31.

33. An at least partially purified preparation of said polyunsaturated fatty acid of claim 31, prepared from a microorganism cultured in accordance with claim 31.

34. A method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism substantially as hereinbefore described with reference to any one of Examples 1, 2 or 3.

ABSTRACT

(Figure 2)

CULTURE OF MICROORGANISMS FOR THE SYNTHESIS OF A POLYUNSATURATED FATTY ACID

C. cohnii is cultured in a suitable growth medium with acetic acid/acetate as the main carbon source. The acetate is provided, and replenished, by adding acetic acid to the growth medium in response to an increase in pH resulting from the utilisation of acetic acid/acetate by the *C. cohnii*. The *C. cohnii* produces relatively high levels of docosahexaenoic acid (DHA). A stationary phase is not essential for satisfactory DHA production.

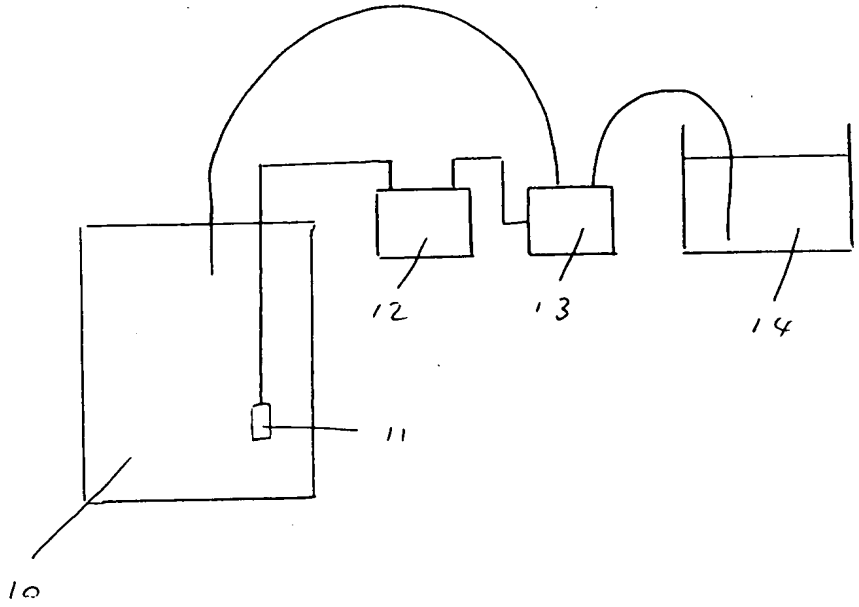
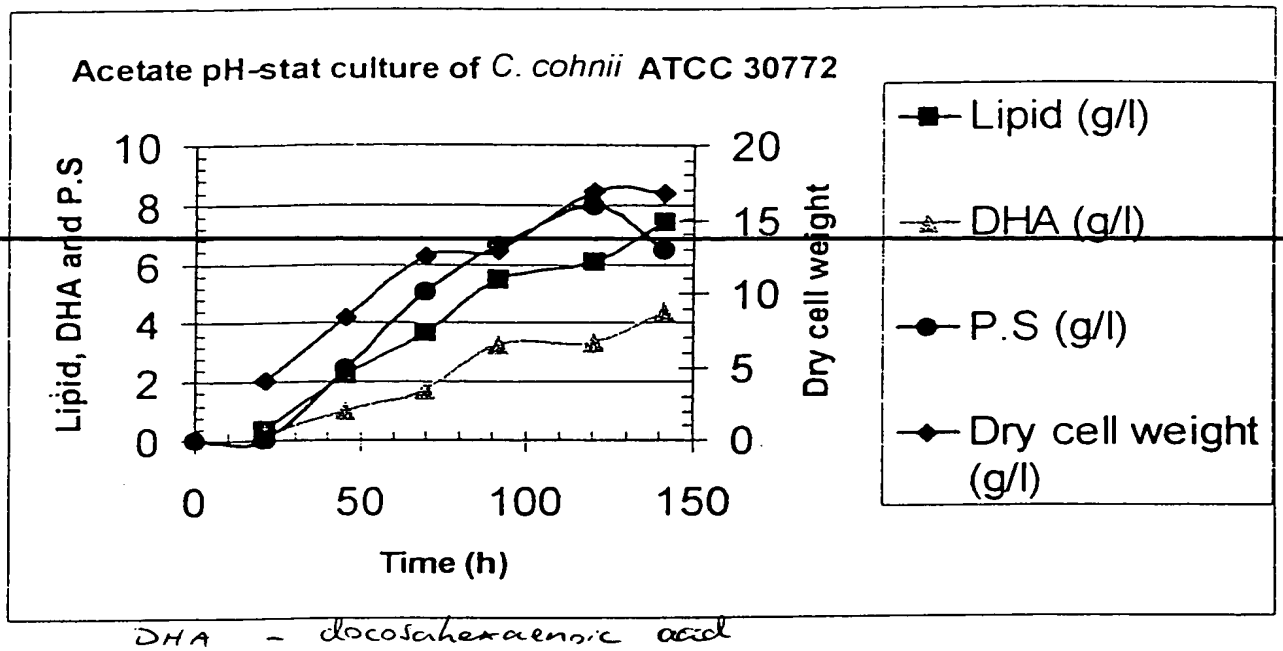


Figure 1

Figure 2



DHA - docosahexaenoic acid
PS - polysaccharide

Figure 3

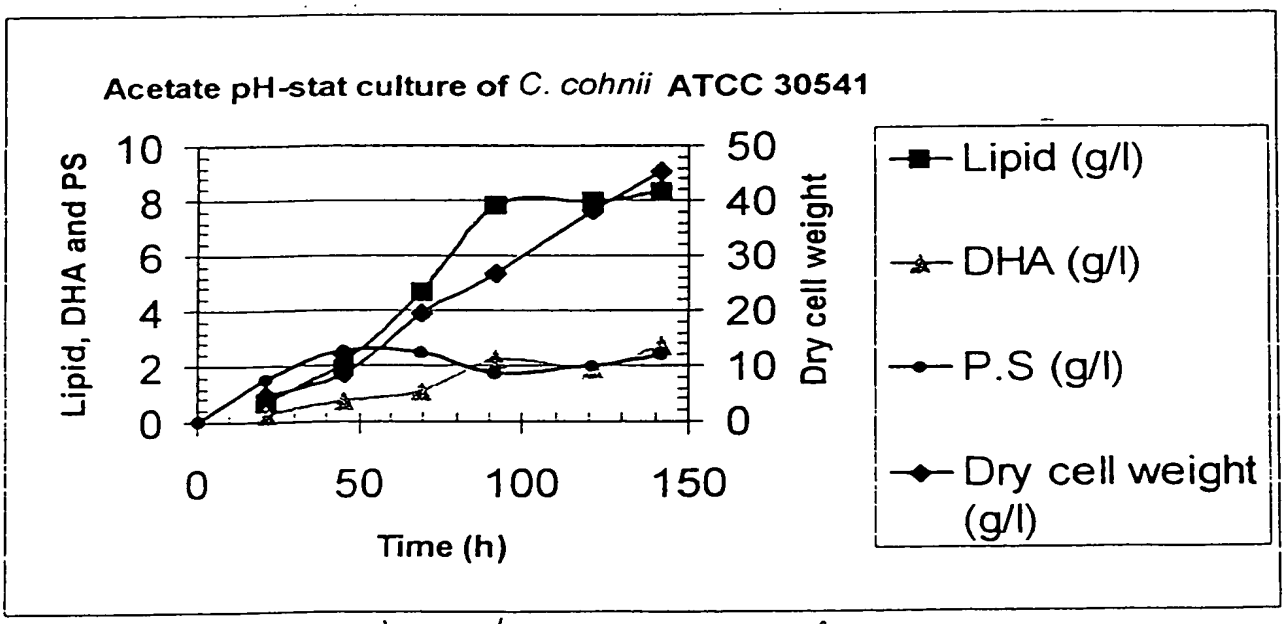


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Figure 4

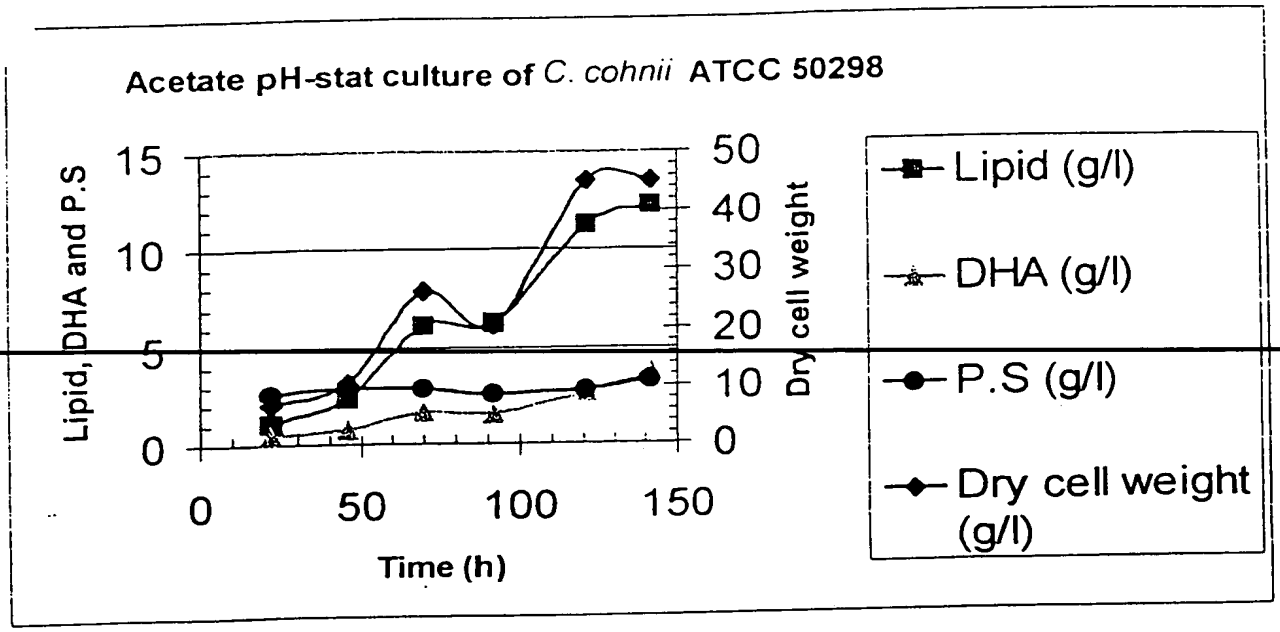

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Figure 5

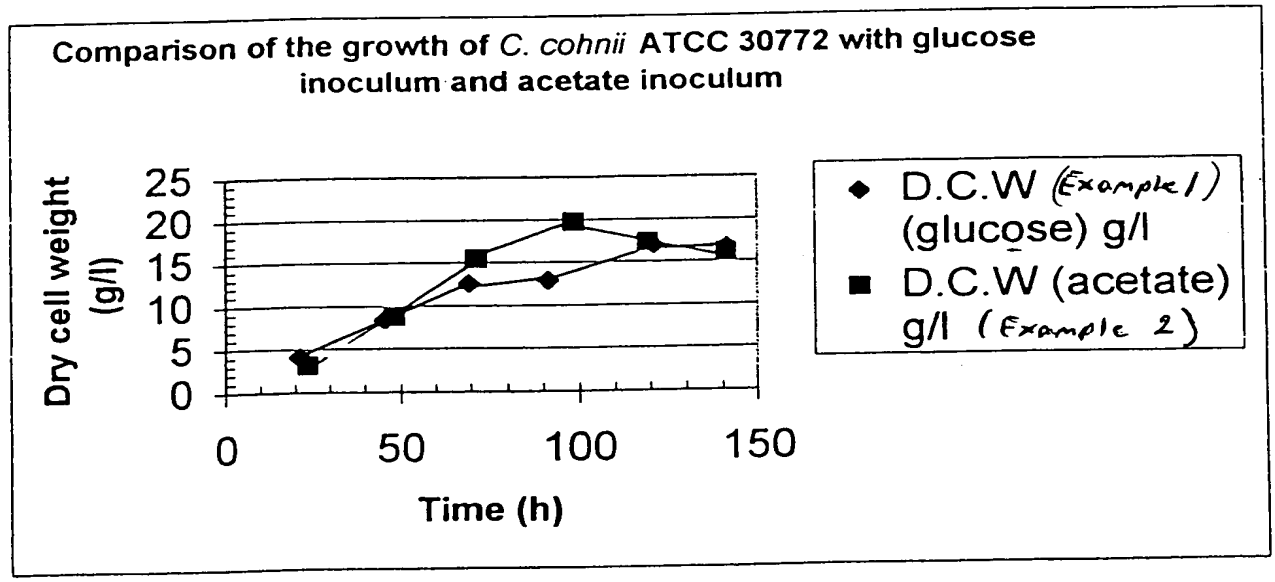
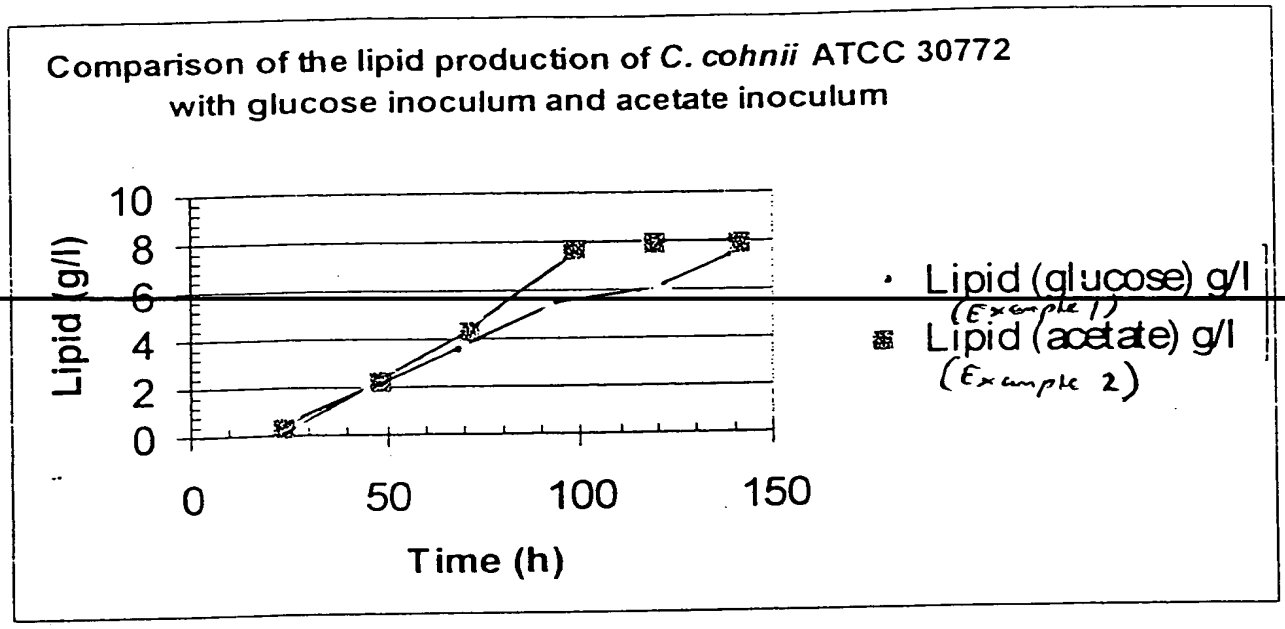


Figure 6



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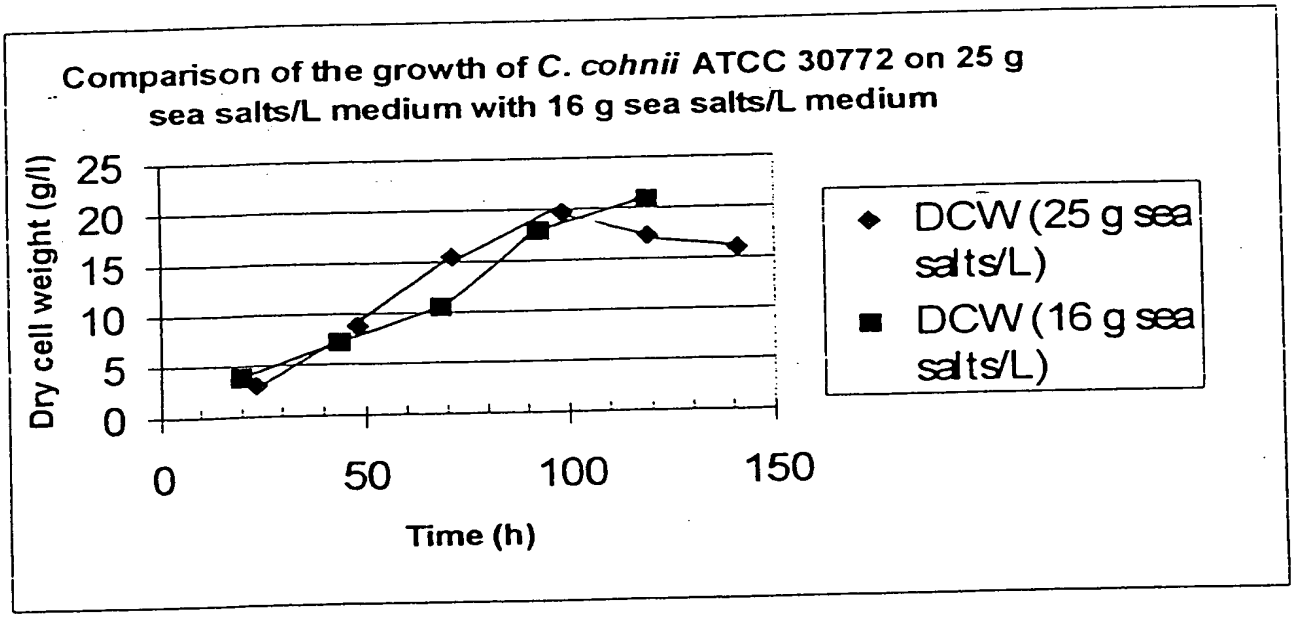

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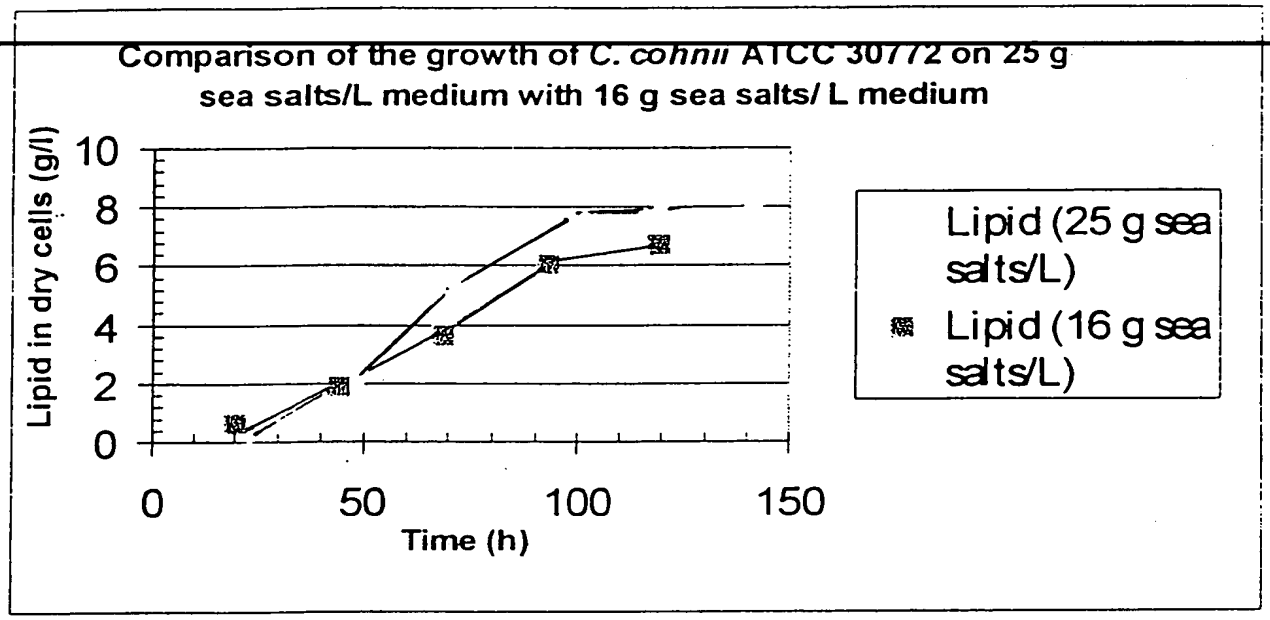

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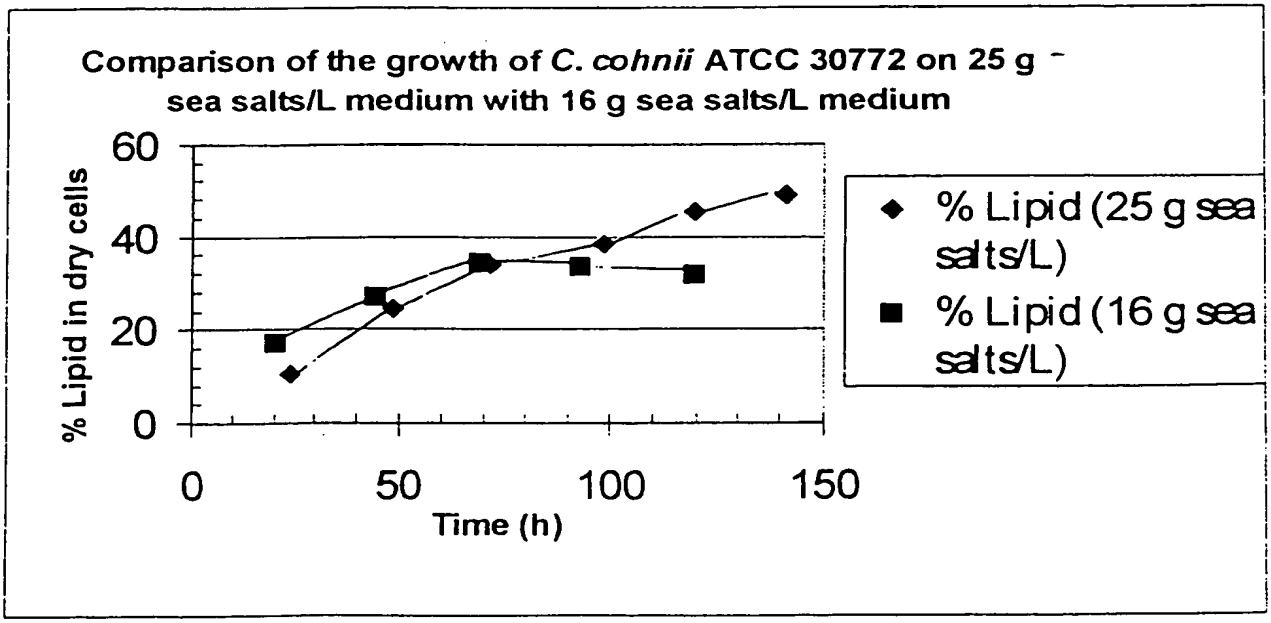

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